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# Evaluation of Four Methods for Rapid Identification of *Staphylococcus aureus* from Blood Cultures

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**The identification of *Staphylococcus aureus* directly from blood cultures is clinically relevant, but it requires a test that is both rapid and reliable. Previously, biochemical, immunological, tube coagulase, and thermostable-endonuclease methods have shown variable sensitivity and specificity. Testing directly from blood culture broth has not been described for the latex kit Staphaurex Plus (Murex Diagnostics Ltd.), and the modified conventional tests have not been used with the newer, continuously monitored blood culture systems. In addition, the commercial RAPIDEC staph kit (bioMerieux Vitek, Inc.) has been used to detect *S. aureus* directly from the Vital blood culture system (bioMerieux, Marcy l'Etoile, France), but its performance has not been evaluated with other continuously monitored systems. A total of 201 clinical blood cultures (BACTEC 9240 culture system; Johnston Laboratories, Inc.) in which a Gram stain showed gram-positive cocci resembling staphylococci were evaluated prospectively. The Staphaurex Plus kit, the tube coagulase test, the thermostable-endonuclease test, and the RAPIDEC staph kit were compared. The sensitivities were 23, 92, 85, and 98% and the specificities were 99, 100, 93, and 100%, respectively. The RAPIDEC staph kit was the most reliable test, with a diagnostic accuracy comparable to that of the best published results for any of the rapid tests. However, it was the most expensive of the tests and relatively labor-intensive. The tube coagulase test was also sensitive, the simplest to perform, and inexpensive.**

The current availability of continuously monitored blood culture systems has reduced the time to detection of positive blood cultures. The use of rapid tests for microbiological identification in concert with the continuously monitored instruments has the potential to greatly reduce the overall time from specimen collection to final identification. A number of rapid tests for identification of *Staphylococcus aureus* directly from blood cultures have been reported. An agar diffusion method for thermostable-endonuclease (TE) detection was found to be an accurate test, with a sensitivity of 96 to 100% and 100% specificity (1, 11). However, other workers have found it less reliable: Davis et al. (3) reported a sensitivity of 68% and a specificity of 93%. Subsequent reports have shown that the accuracy of the test is dependent on the constituents of the blood culture system and the DNase medium used (2, 4).

The traditional method for differentiating *S. aureus* from coagulase-negative staphylococci (CNS) in the laboratory has been the tube coagulase test (TCT). This test has been modified for direct detection of the organism from blood cultures in 2 h by using the blood culture broth. Davis et al. (3) found the test sensitive (97%) and specific (100%), but more recently McDonald and Chapin (7) reported a lower sensitivity, 79.5%.

Immunological tests for the rapid identification of *S. aureus* from blood cultures have been more frequently reported. The Staphaurex test has been evaluated in several studies (3, 7, 10, 12) and found to have generally poor sensitivities (12 to 80%). More recently, the test Staphaurex Plus (Murex Diagnostics Ltd.) has been marketed; this kit recognizes a somatic and a capsular antigen, in addition to clumping factor and staphylococcal protein A, as detected by the original Staphaurex kit.

Biochemical tests have also been used for direct detection of *S. aureus* from blood cultures. The AutoMicrobic system Gram-Positive Identification Card (Vitek Systems, Inc., Hazelwood, Mo.) was found to be insensitive (6), but the RAPIDEC staph kit (bioMerieux Vitek, Inc.) has been reported to be much more reliable. The latter kit was specifically designed to detect *S. aureus* in Vital system blood cultures (9). The kit identified 27 of 28 *S. aureus* isolates in clinical blood cultures with 100% specificity with the BACTEC NR-660 and Oxoid SIGNAL systems (8) and all 35 *S. aureus* isolates with 98.8% specificity with the Vital system (9).

To our knowledge none of the rapid tests has been applied to the BACTEC 9240 continuously monitored blood culture system. In addition, the Staphaurex Plus test and the RAPIDEC staph kit have not been prospectively compared to the previously described tests. By using the BACTEC 9240 (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) blood culture system, a direct prospective comparison of the TCT, the TE test, the Staphaurex Plus kit, and the RAPIDEC staph kit was conducted.

## MATERIALS AND METHODS

**Positive-blood-culture processing.** Blood for cultures was collected according to clinical criteria. Blood collected from patients was inoculated into aerobic and anaerobic BACTEC 9240 bottles at the bedside and then incubated at 37°C for 5 days. Bottles with a positive growth index were removed, and a Gram stain was performed. Broths for which a Gram stain showed gram-positive cocci resembling *Staphylococcus* spp. were included in the study. *Staphylococcus* spp. isolated from solid-medium blood cultures were identified by standard laboratory methods which were the "gold standard" against which the sensitivity and specificity of the rapid tests were compared.

**Rapid tests.** Several methods were employed for the rapid (2-h) identification of *S. aureus*. For the TCT, 100 μl of well-mixed broth was inoculated into 0.5 ml of rabbit plasma (BBL Microbiology Systems) and the mixture was incubated at 37°C for 2 h. The test was scored as positive if a clot formed. Tubes with a negative result were incubated overnight and read again the next day.

For the TE test, an agar diffusion method was adapted from the method of Bergh and Maeland (1). A 2-ml aliquot of blood culture broth was boiled for 15 min and then cooled to room temperature. Six-millimeter holes were cut in

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TABLE 1. Gram-positive cocci suggestive of staphylococci isolated from 201 blood culture specimens

| Organism(s) | No. of isolates (%) |
|---|---|
| *S. aureus* | 58 (29) |
| Mixed (*S. aureus* and CNS) | 2 (1) |
| Methicillin-sensitive *S. aureus* | 43 (72) |
| Methicillin-resistant *S. aureus* | 17 (28) |
| CNS | 141 (70) |
| Total | 201 (100) |

methyl green DNase agar (GIBCO Laboratories), and 60-μl volumes of the fluid phase of the broth were transferred to the wells. The plates were incubated at 37°C for 2 h, and then TE activity was detected by the presence of a clear zone with a darker green line surrounding the well. Cultures of *S. aureus* and *S. epidermidis* were included as positive and negative controls, respectively.

For immunological identification of *S. aureus*, the Staphaurex Plus test was modified for use with the bacterial pellet. The pellet was obtained by centrifuging 4 ml of broth at 150 × *g* for 10 min to sediment the erythrocytes. The supernatant was then centrifuged at 1,000 × *g* for 10 min to concentrate the bacteria, and the latex test was performed on the bacterial pellet according to the manufacturer's instructions.

The RAPIDEC staph test was performed according to the manufacturer's instructions with an additional step to remove the erythrocytes. Briefly, 3 ml of broth was centrifuged (150 × *g* for 10 min) to sediment the erythrocytes and then 2 ml of the supernatant was mixed with 2 ml of distilled water and centrifuged at 1,000 × *g* for 10 min to produce a bacterial pellet. The pellet was then resuspended in distilled water to provide a 4 McFarland turbidity equivalent, and 50 μl was added to cupules 0 and 1 (the "aurease" control and test, respectively). The strip was then incubated at 37°C for 2 h, and the fluorescent reaction was examined under UV light (365 nm). The test was scored as positive when the test well was more fluorescent than the control well.

## RESULTS

A total of 201 blood cultures in which a direct Gram stain showed gram-positive cocci resembling staphylococci were examined by commercial immunological and biochemical tests and rapid modifications of conventional tests (Table 1). These blood cultures were obtained over a 5-month period from April to August 1997 at the Clinical Microbiology Laboratories, Institute of Clinical Pathology and Medical Research, Westmead, Australia. Of the isolates from the blood cultures, 70% were CNS and 30% were *S. aureus*; 28% of the *S. aureus* isolates were methicillin resistant. Two blood cultures contained both *S. aureus* and CNS.

The results are summarized in Table 2. All tests were performed prospectively without knowledge of the final microbiological identification. The results for methicillin-resistant *S. aureus* were not significantly different from those for methicillin-sensitive *S. aureus*. The Staphaurex Plus was the least sensitive of the tests, detecting only 23% of the *S. aureus* isolates. The TE test was moderately sensitive (85%) but demonstrated the lowest specificity (93%). The TCT was sensitive (92%) and specific (100%), but the RAPIDEC staph test was the most sensitive (98%), highly specific (100%), and hence the most reliable test overall. TCTs that were false negative at 2 h were all positive after overnight incubation. The TCT, the TE test, and the RAPIDEC staph test were all positive for 48 (80%) of the *S. aureus* isolates. The TE test was positive for three of the five false-negative TCTs, and the TCT was positive for seven of the nine false-negative TE tests. The RAPIDEC staph test was always positive when the TCT was positive. One blood culture containing both CNS and *S. aureus* gave negative results with all the tests. The positive predictive value of the TCT and the RAPIDEC staph test was 100%, and these tests had the highest negative predictive values, 97 and 99%, respectively.

The combined material and labor costs of the tests are also shown in Table 2; the TCT was the least, and the RAPIDEC staph kit was the most, expensive.

## DISCUSSION

The use of rapid direct tests with blood cultures has been shown to be clinically relevant, prompting the initiation of antibiotic therapy or a change to more appropriate antibiotic therapy (13). The results of our study suggest that rapid direct tests on blood cultures can identify *S. aureus* with a degree of accuracy that allows such clinical decisions to be made.

As previously shown for other immunological latex kits used with other blood culture systems, we found the Staphaurex Plus kit to be neither sensitive nor specific when used with the BACTEC 9240 system. Several methods of inoculum preparation for rapid identification by the latex kits have been tried previously, with poor results (3, 7, 10). We had thought the new Staphaurex Plus kit, with the added capability of detecting somatic and capsular antigens, might yield superior results. Unfortunately, our results confirmed those previously published, suggesting that immunological latex tests such as the Staphaurex Plus kit have no role in rapid detection of *S. aureus* from blood cultures.

A 2-h TCT was found to be superior to the immunological tests by Davis et al. (3), reporting a sensitivity of 97% and a specificity of 100% when the TCT was performed directly with blood culture broth, but a similar, more recent, study (7) showed only moderate sensitivity (79.5%). It was suggested that the lower sensitivity may have been due either to differences in the interpretation of weakly positive results or to variation in batches of rabbit plasma from different suppliers. We found the TCT to be sensitive and specific, simple to perform, and relatively inexpensive.

The TE test has been reported by some workers to be suitable for rapid identification of *S. aureus* (1, 12), with excellent sensitivity and specificity within 2 h, but others have not reproduced these results. Davis et al. (3) used the test with bacterial pellets and directly on blood culture broth, with sensitivities of 68 and 100% and specificities of 93 and 89%, respectively. We

TABLE 2. Results and costs of rapid tests for direct identification of *S. aureus* from blood cultures

| Direct test | No. of cultures positive/no. containing *S. aureus*[a] (% sensitivity) | No. of cultures negative/no. containing CNS (% specificity) | Positive predictive value (%) | Negative predictive value (%) | Cost/test (US$) |
|---|---|---|---|---|---|
| Staphaurex Plus | 14/60 (23) | 139/141 (99) | 87 | 75 | 6.63 |
| TE | 51/60 (85) | 131/141 (93) | 84 | 94 | 7.77 |
| TCT | 55/60 (92) | 141/141 (100) | 100 | 97 | 4.37 |
| RAPIDEC staph | 59/60 (98) | 141/141 (100) | 100 | 99 | 9.91 |

[a] *S. aureus* in pure or mixed culture.

also found the test less reliable. It has been shown that the test is extremely medium dependent, with the source of the DNase agar being particularly important (4). False-positive results with other *Staphylococcus* species have also been reported (2). In addition, the test requires a boiling step, making it more complicated than the TCT.

The most sensitive of the tests was the RAPIDEC staph test, with 98% sensitivity and 100% specificity. When isolates from solid media (5) and blood cultures (8) were tested with other culture systems, 100% specificity was found; however, one false-positive result was reported for the Vital system (9). This was thought to be due to excessive hemolysis, a problem known to cause false-positive results (11). We found one false-negative result from 60 *S. aureus* isolates. This isolate, which gave negative results with all the rapid tests, was from one of two blood cultures in which a mixed growth of *S. aureus* and CNS was found. This would have resulted in a smaller-than-recommended inoculum of *S. aureus* in the test well of the RAPIDEC staph kit. When the RAPIDEC staph test was repeated with the blood culture broth the next day, the result was positive, suggesting that the initial inoculum was insufficient. In another study in which blood culture broths were tested, one false-negative result was found among 28 *S. aureus* isolates; other investigators have reported 100% sensitivity (9). The test was relatively time-consuming, requiring two centrifugation steps, and was the most expensive.

The concordance between the TCT and the RAPIDEC staph test was expected, as both tests detect coagulase; the RAPIDEC staph was the more sensitive at 2 h. In contrast, the TE test and the TCT, which detect different enzymes, did not show the same association. The Staphaurex Plus test was too insensitive to allow comparison with the other rapid tests.

In summary, we compared modified conventional tests and commercial tests not previously assessed for their ability to directly detect *S. aureus* from the BACTEC 9240 continuously monitored blood culture system. The RAPIDEC staph test was the most sensitive but also the most expensive. The rabbit plasma TCT was also sensitive, second only to the RAPIDEC staph kit; the simplest to perform; and the least expensive. The TCT and the RAPIDEC staph test had a positive predictive value of 100%; thus, with a positive test the significance of the isolate could be confirmed within several hours of the positive blood culture, avoiding delays in antimicrobial therapy for *S. aureus* bacteremia. However, since neither of these tests had 100% negative predictive value, a negative test would dictate treatment based on clinical grounds until further identification of the isolate. Following this study, the TCT performed with blood culture broth has been adopted as the routine test in our laboratory. Positive results are reported immediately as *S. aureus*, whereas negative results are reported as "gram-positive cocci suggestive of staphylococci" until conventional identification and reading of the TCT the following day.

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